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Microbiome of HIV-infected people

Grégory Dubourg [a, b, *], Mathieu Surenaud [c, d, e], Yves Lévy [c, d, e, f], Sophie Hüe [c, d, e, f], Didier Raoult [a, b]

[a] Pôle des Maladies Infectieuses et Tropicales Clinique et Biologique, Fédération de Bactériologie-Hygiène-Virologie, University, Hospital Centre Timone, Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, Assistance Publique - Hôpitaux de Marseille, Marseille, France
[b] Université Aix-Marseille, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE) UM 63 CNRS 7278 IRD 198 INSERM U1095, Facultés de Médecine et de Pharmacie, Marseille, France
[c] Inserm, U955, Equipe 16, Créteil, 94000, France
[d] Université Paris Est, Faculté de médecine, Créteil, 94000, France
[e] Vaccine Research Institute (VRI), Créteil, 94000, France
[f] AP-HP, Hôpital H. Mondor - A. Chenevier, Service d'immunologie biologique, Créteil, 94000, France

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ABSTRACT

Consistent interactions between the gut microbiome and adaptive immunity recently led several research groups to evaluate modifications of human gut microbiota composition during HIV infection. Herein we propose to review the shifts reported in infected individuals, as their correlation to disease progression. Though the gut microbiota is consistently altered in HIV individuals, the literature reveals several discrepancies, such as changes in microbial diversity associated with HIV status, taxa modified in infected subjects or influence of ART on gut flora restoration. Similarly, mechanisms involved in interactions between gut bacteria and immunity are to date poorly elucidated, emphasizing the importance of understanding how microbes can promote HIV replication. Further research is needed to propose adjuvant therapeutics dedicated to controlling disease progression through gut microbiome restoration.

Contents

* Corresponding author. URMITE, UMR CNRS 7278, IRD 198, INSERM U1095, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille cedex 5, France.
E-mail address: greg.dubourg@gmail.com (G. Dubourg).

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1. Introduction

Humans harbour nearly 100 trillion intestinal bacteria that are essential for health. These microorganisms establish symbiotic relationships with their hosts, making essential contributions to mammalian metabolism while occupying a protected, nutrient-rich environment. The relationship between the host and its microbiota is described as a superorganism that plays an essential role in maintaining health and immunity. Its interplay with the immune system has been demonstrated over the past decade, highlighting its involvement in several infectious diseases. Indeed, loss of bacterial diversity induced by antibiotics is well-known to be associated with *Clostridium difficile* infections [1,2]. Also recently, the same low diversity was shown to be a predictive factor of *Camplylobacter* infections [3], independently of antibiotic treatment. Above all, the gut microbiome is a significant source of immunological interactions whose mechanisms are currently only partially known. As an example, the gut flora acts as an adjuvant that promotes influenza vaccination through co-stimulation by microbiota-released flagellin [4]. Complex interactions between microbes constitute a barrier against enteric pathogens as has been demonstrated for *Salmonella typhimurium* colonization [5] or through production of antimicrobial molecules [6]. Experiments performed in germ-free mice infected with *Klebsiella pneumoniae* showed that these animals are highly susceptible to bacterial infection in an IL-10-dependent manner. Thus, Lipoplysaccharide (LPS) pretreatment also rendered germ-free mice resistant to pulmonary *K. pneumoniae* infection. These results suggest that symbiotic gut colonization enables proper inflammatory response to harmful insults to the host [7]. In the same way, the presence of segmented filamentous bacteria within gut microbiota is associated with protection against *Staphylococcus aureus* pneumonia [8]. The role of Gram-negative bacteria from gut microbiota in immune activation and chronic inflammation in HIV individuals is known since circulating LPS had been used as an indicator of microbial translocation [9]. In this context, the possible role of the intestinal microbiome in disease progression had led several teams to assess changes in gut microbial communities during HIV infection. In this review, we have mainly reviewed articles focusing on gut flora compositional disruptions observed in infected subjects, as possible mechanisms involved in disease progression. We also provide perspectives as first conclusions from recent therapeutic strategies dedicated to restoring the gut flora in HIV-subjects.

2. Gut microbiota composition and susceptibility to infections of mucosal origin

Among opportunistic infections, HIV-related disease had long been associated with bacteremia, especially before the existence of antiretroviral therapy (ART). The spectrum of microorganisms seemed to be specific to HIV infection. Thus, *Streptococcus pneumoniae* and *Salmonella* spp. were the organisms the most frequently associated with seropositive status [10]. While immunological status, such as CD4 T-cell counts, had been shown to play a role in the occurrence of invasive pneumococcal diseases (IPD) [11], this is unclear for *Salmonella* bacteremia for which risk factors have not been elucidated [12]. As a matter of fact, even though its incidence is raised among men who have sex with men (MSM), HIV status had been shown in a case control study to increase host susceptibility to *Shigella* infections [13]. This raises the question of gut mucosal microbiota involvement. Indeed, recent studies had shown that low oxygen tension in gut tissue promotes *Salmonella* multiplication, and increases its virulence. Besides, its virulence effectors play a role in intra-species dynamics for colonization [14]. By extension, invasive pneumococcal disease had been associated with modifications of the airway microbiome. These data support the claim that the mucosal microbiota and its interactions with the immune system could facilitate invasion of infectious agents from mucosal surfaces. This should be contrasted with a recent report, in which desertification of the gut microbiota induced by antibiotics from a patient suffering from XDR-tuberculosis had been associated with fatal sepsis due to IPD [15]. Considering recent advances in understanding the pathophysiological and immunological mechanisms associated with HIV disease progression, these findings led to a focus on gut microbiota composition disruptions in HIV-infected subjects.

Gut microbiota analysis

For many years, culture techniques were time the only ones available to assess gut microbiota composition. Since the advent of molecular techniques, in particular high-throughput sequencing technologies, our knowledge of the bacterial repertoire has exploded, permitting rapid, reproducible and cost-effective comparison between large cohorts of different specimens. However, despite significant advantages, these methods exhibit several pitfalls from sample collection to data interpretation. As a matter of fact, microbial signatures differ depending on whether samples are mucosal biopsies, feces or rectal swabs [16,17] and these discrepancies were highlighted in studies dedicated to the HIV gut microbiota. These findings raise the question about the appropriate specimen to collect for studying gut flora composition and in particular its interactions with the immune system, for which adherent mucosal microorganisms are largely involved. Extraction bias has also been extensively reported, from sample lysis to DNA purification. Thus, a heating step at 95 °C, combined with repeated silica bead beating is currently considered as the reference method for releasing DNA [18,19]. Altogether, the use of commercial kits to extract nucleic acids is tempting to improve workflow, but induces multiple significant biases [20–23]. While the 16S approach is the most commonly used, several limitations are now well-established. Thus, the copy number of the 16S rDNA gene fluctuates among bacterial species, and among strains within the same species [24–26], leading to under- or overestimation of relative abundances [27]. In addition, the choice of the targeted hypervariable region is crucial, as choice of the V1–V3 region results in higher richness than when V3–V5 is selected, but is unable to detect Bifidobacteria [28]. Finally, the inability of the high throughput sequencing technologies to detect microorganisms at concentrations lower than 10^5/mL reflects the depth bias [29].

3. Gut microbiota and immune homeostasis

Homeostasis in the gut mucosa is maintained by a system of checks and balances between potentially pro-inflammatory cells, including Th1 cells that produce interferon-γ, Th17 cells that produce IL-17A, IL-17F, and IL-22 and anti-inflammatory $Foxp3^+$ regulatory T cells (Tregs). Th17 cells play a major role in both epithelial homeostasis and host defense against various extracellular pathogens such as *Candida albicans* and *Pseudomonas aeruginosa.* IL-17 and IL-22 stimulate the production of antimicrobial proteins (AMP) by the epithelium and thereby sustain its barrier function. They also induces the recruitment of neutrophils that eliminate bacteria having translocated across epithelium. Although Tregs and Th17 cells exert mostly opposing functions, these cells are two closely related CD4 T-cell subsets sharing reciprocal maturation pathways [30]. There is an active balance between the development of either Tregs or Th17 cells and even plasticity between the two subsets [31]. It has recently become evident that individual commensal species influence the balance between these T lymphocyte subsets. For example, polysaccharide-A (PSA) of *Bacteroides fragilis* has been shown to induce IL-10 expression in